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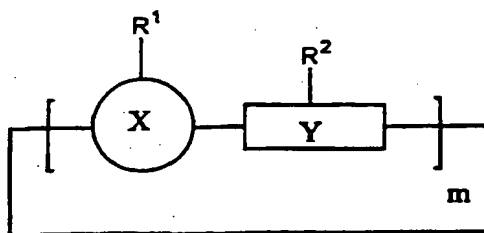
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(I)

(57) Abstract: Especially for use in the capture of a solute in solution, a macrocyclic compound has the formula (I) wherein X is a ring bearing an optionally-protected functional group or solid phase group R<sup>1</sup>, Y is a linker optionally bearing an optionally-protected functional group or solid phase group R<sup>2</sup>, m is at least 3, and each instance of X, Y, R<sup>1</sup> and R<sup>2</sup> may be the same or different.

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## MACROCYCLIC COMPOUNDS AND THEIR USE

### Field of the Invention

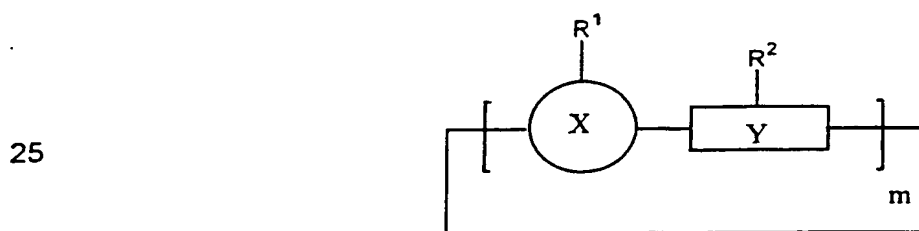
The invention relates to macrocyclic compounds and their use.

### Background of the Invention

5        There is a growing interest in macrocyclic molecules that may be used as scaffolds in the combinatorial synthesis of receptor molecules. Many macrocyclic molecules have been synthesised. However, in many cases, their synthesis is difficult and/or relatively inflexible towards functionalisation. For some recent examples of unsymmetrically substituted macrocycles, see Rasmussen *et al*, J. Tet. Lett. (1999) 40: 10    3511; Höger *et al*, Chem. Eur. J. (1999) 5: 1686; Shu *et al*, J. Org. Chem. (1999) 64: 2673; and Cho *et al*, Bioorg. Med. Chem. (1999) 7: 1171. Cho *et al* discloses cyclic and linear oligocarbamates. Only in the case of cyclic peptides have stepwise synthesis, facile functionalisation of the building blocks, and solid phase synthesis been reported.

      Some known macromolecules are based on triazine. Oligomers and cyclic  
15    molecules of this type are disclosed by Lipkowski *et al*, Chem. Commun. (1999) 1311; Ichihara *et al*, Chem. Letters (1995) 631; Mathias *et al*, J. Am. Chem. Soc. (B1994) 116: 4326; and Anelli *et al*, J. Org. Chem. (1984), 49: 4197. In particular, Mathias *et al* discloses linear triazine-based macromolecules, capable of supramolecular aggregation. Ichihara *et al* discloses triazine-linked porphyrins. Lipkowski *et al*  
20    discloses compounds that form a [2x2] hydrogen-bonded grid.

      Known macrocycles include those of the formula



      where X is a ring which may bear a group R<sup>1</sup>, Y is a linker which optionally bears a group R<sup>2</sup>, and m is an integer. Thus, Rasmussen *et al* discloses compounds in which  
30    X is a benzene ring, R<sup>1</sup> is H, Y is peptidic, R<sup>2</sup> is a functional group, and m is 3, for combinatorial applications. Anelli *et al* discloses compounds in which X is triazine, R<sup>1</sup> is Cl and m is 2, as intermediates to triply-bridged derivatives, capable of molecular recognition and of use as phase-transfer catalysts.

### Summary of the Invention

The present invention is based on a stepwise approach to the combinatorial synthesis of macromolecules having a desired/defined stereochemistry and also functional groups effectively predefined both for the potential uses of the molecule and for its synthesis (which can be done on a solid phase).

According to a first aspect of the present invention, compounds are of the formula given above, wherein X is a ring bearing an optionally-protected functional group  $R^1$ , Y is a linker optionally bearing an optionally-protected functional or solid phase group  $R^2$ , and m is at least 3; each of X, Y,  $R^1$  and  $R^2$  may be the same or different.

Compounds of this invention provide, typically by choice of the functional group  $R^1$ , an effective recognition element. Depending on the choice of groups  $R^1$ , compounds of the invention have a variety of uses, for example depending on their binding properties, for diagnostics and therapeutics. They may be used for affinity binding, phase-transfer agents and ionophors. Compounds of the invention may also be used for molecular imprinting and for other purposes such as removing pesticides from water and for high performance chromatography. In a particular example, compounds of the invention may be constructed to recognise portions of proteins, and thereby have value for targeting or separation purposes. Their utility may also depend on characteristics of the molecule other than  $R^1$ , e.g. the value of m (which determines the "hole" in which foreign molecules may be bound). The characteristics of compounds of the invention may be extended by using them in a multi-layer format.

### Description of the Invention

X is preferably triazine. It may also be any ring that bears or can readily be reacted to form the three given substituents on X, i.e.  $R^1$  and two linkers Y. Y may be any suitable linker, of any desired length; it will often be preferred that the molecule forming the linker terminates in a functional groups such as NH, which can readily be reacted with a carbon atom in the ring X. By this means of synthesis, any group  $R^1$  or  $R^2$  may already be present on X/Y. This allows a wide selection of suitable functional groups, according to the desired end purpose. Suitable protecting groups, their introduction and removal, are well known to those in the art, and will be used as necessary for protection during synthesis. The primary purpose of the functional group  $R^1$  will be with respect to foreign molecules to be bound by macrocycles of the invention; for this purpose, the groups  $R^1$  may be the same or different.  $R^1$  may also be used as a point of attachment to a solid phase.  $R^2$  may be absent; if present, it will

generally be chosen with a view to ready immobilisation of the linker, to facilitate synthesis and/or use of the macrocycles. As indicated below, this invention allows effective solid phase chemistry to be used.

m will typically be 3. It may however be a higher integer, e.g. 4, 5 or 6,  
5 depending on the desired size of the macrocycle.

The solid phase may be any material that may conveniently be used to separate macrocyclic compounds of the invention from solutes in a contacting solution. Examples of suitable solid phase support materials include, but are not limited to, polysaccharides such as agarose, cellulose, dextran, starch, alginate and carrageenan;  
10 synthetic polymers such as polystyrene, styrene-divinylbenzene copolymers, polymethacrylates, (e.g. poly(hydroxyethylmethacrylate), polyvinyl alcohol, polyamides, polyacrylamides and perfluorocarbons; inorganic materials such as glass, silica and metal oxides; and composite materials.

A variety of chemistries have been developed for the attachment of affinity  
15 ligands to solid phases. It will be appreciated by those skilled in the art that any convenient linker chemistry may be used as a means of attachment of macrocyclic compounds of the invention to a solid phase. Such linker chemistry may include both cleavable and non-cleavable groups. It will also be appreciated by those skilled in the art that macrocyclic ligands of the invention may be attached to a solid phase on  
20 completion of their synthesis or built up on the surface of a solid phase in step-wise fashion by solid phase synthesis. It will also be evident to those skilled in the art that libraries of macrocyclic compounds of the invention with differing X, Y, R<sup>1</sup> and R<sup>2</sup> groups may be constructed and subsequently screened for binding activity towards target compounds.

25 Macrocyclic compounds of the invention have general utility for the capture of complementary molecules. It will be appreciated that such compounds have many potential applications. Applications of compounds of the invention include, but are not limited to, the analysis, separation, isolation, purification, quantification, characterisation, or identification of chemical, biological or therapeutic compounds, the  
30 diagnosis of disease, and the treatment of disease. For example, compounds that can be separated from admixture with others include organic heterocycles such as cyanuric acid and saccharide-containing compounds such as octylglucosides. Examples of therapeutic application are in the treatment of immune disorders and metabolic disorders by capture or clearance of carbohydrates and glycoproteins, and the removal  
35 of toxic substances from blood.

For the purposes of illustration only, and with reference to Scheme 1, the invention will now be described with reference to the synthesis of macrocycles based on the triazine unit as a building block, with each unit comprising a triazine ring and a linker moiety (piperazine or diamine). In each stage of the synthesis, the chain can be either elongated or cyclised to the macrocycle of interest. The use of two orthogonal protective groups ( $P^1$ ,  $P^2$ ) on either side of the oligomers, allows control of the length of triazine-piperazine chain. In addition, it is possible to functionalise each triazine unit in the chain differently by reaction with particular amines. In Examples 1 and 2, piperazine was used as the linker, in order to create relatively rigid macrocycles. It will be evident to those skilled in the art that larger rings may be prepared and that other linkers may be used, thereby increasing the diversity even further.

The following Examples illustrate the invention. Reference may also be made to the accompanying Schemes 2 to 4.

**Example 1 Preparation of macrocyclic triazine compounds in solution**

The synthesis of the basic macrocyclic structures commenced with mono-Boc protection of piperazine 1 using a modification of the procedure described by Carpino *et al*, J.Org. Chem (1983) 48:661. Reaction of monoprotected piperazine 2 with cyanuric chloride gave the monosubstituted product 3 in 93% yield, using the procedures of Koopman *et al*, J. Rec. Trav. Chim. Pays-Bas (1958) 77:235 and Beech, Chem. Soc. C. (1967) 466. Compound 3 can be used throughout the synthesis as a convenient building block for the elongation of triazine-piperazine oligomers. Mono-Z protected piperazine was chosen as the second substituent on the triazine 3, since it provided a second piperazine moiety, orthogonally protected. The mono-Z-protected piperazine was obtained by subsequent reaction of 2 with Z-chloride and TFA to give a quantitative yield of piperazine 5. This route is convenient and gives high yield. In addition, the reaction of piperazine with Z-chloride only gave the bisubstituted product.

The reaction of Z-protected compound 5 with 3 afforded bisubstituted triazine 6 in 85% yield. Finally, the third substituent (amylamine) was introduced by refluxing 6 with an excess of amylamine to give trisubstituted triazine 7a in 99% yield; see Li *et al*, J. Org. Chem. (1996) 61:1779. Diversity can be achieved by varying the amine in this step; other model amines used included benzylamine, cyclohexylamine, dodecylamine, 2-phenylethylamine and aniline, all of which gave trisubstituted products.

7b-d were obtained in 98%, 91% and 85% yield, respectively. These reactions show that a great variety of amines may be used in this reaction, to provide the desired

diversity. The triazines **7** are also the starting point for the preparation of a series of functionalized triazine-piperazine oligomers that can be eventually cyclised. At this point, compound **7a** was chosen to continue the work: either the Boc or the Z-group could be removed to functionalise further this compound. Thus, in order to obtain the precursor for the synthesis of a bis-triazine macrocycle, compound **7** was subjected to subsequent hydrogenolysis and a reaction with cyanuric chloride to afford dichloride **8** in a yield of 43%. Preliminary results revealed that higher yields of macrocycles could be obtained if the last protective group before cyclisation was a Boc group rather than a Z-group. Alternatively, compound **7** was treated with TFA, followed by a reaction with dichloride **3**, using triethylamine as a base to give a quantitative yield of bis-triazine **9**. The remaining chloride was then substituted using an excess of isobutylamine to afford compound **10** in 93% yield. Removal of the Z-group and reaction with cyanuric chloride gave dichloride **11** in 61% yield, as a precursor for the synthesis of a tris-triazine-piperazine macrocycle. Elongation of bis-triazine **10** was obtained by subsequent reaction with TFA and building block **3** to produce tris-triazine **12** in a yield of 93%. The remaining chlorine was displaced with cyclohexylamine to give tris-triazine **13** in 92% yield. Repetition of these steps also afforded the precursors for the tetra-, penta- and hexa-triazine-piperazine macrocycles **14**, **17** and **20** in 62%, 46% and 43% overall yields, respectively, from compound **13**.

The triazine-piperazine oligomers **8**, **11**, **14**, **17** and **20** were used to prepare their respective macrocycles by subsequent treatment with acid and base (scheme 4). All compounds, except **8**, could be converted to their corresponding macrocycles in fair yields (59-83%). Compound **8** produced only the dimer (tetra-triazine **21**), while for the others, dimers were not obtained under the reaction conditions employed. Finally, the remaining chlorines in **22**, **23**, **24** and **25** were substituted using an excess of benzylamine to give **26**, **27**, **28** and **29**.

#### **Example 2 Preparation of immobilised macrocyclic triazine compounds by solid phase synthesis**

Having shown that it is possible to synthesise macrocycles based on triazine-piperazine building blocks in solution, the next step was to repeat this or similar chemistry on a solid phase. Synthesising the macrocycles on a solid phase allows the possibility to generate many variants that may be screened easily for their binding properties. However, in order to attach the compounds to a resin, appropriate coupling chemistry or a 'handle' is required. A flexible way to achieve this without having to use

one of the triazine rings was to use an additional functionality on one of the piperazine rings, as found in piperazine 30. It was also considered beneficial to have a linker between the resin and the triazine-piperazine based molecules, in order to permit detachment from the solid phase and analysis of their structure. This may be achieved by inserting a methionine residue between the piperazine 30 and the resin, in order to give a linkage that is stable to the conditions (acids, bases and nucleophiles) under which the macrocycles are synthesised. The methionine linkage may be subsequently cleaved with cyanogen bromide. Alternatives include a photocleavable bond or one involving a vicinal diol and cleavable by periodate oxidation,. Furthermore, instead of building block 3, N-protected triazine-piperazine 32 was used, in which a trityl protective group is used such that it may be removed without cleaving a Boc group. Compound 32 is easily synthesised in the two steps shown in Scheme 5, in a 58% overall yield.

The solid phase synthesis was initiated by attachment of methionine and piperazine 30 using Fmoc chemistry and BOP as a coupling agent (Scheme 6). Cleavage using cyanogen bromide, after removal of the Fmoc and Boc groups and subsequent capping with  $\text{Ac}_2\text{O}$ , gave compound 35. Alternatively, removal of the Fmoc group and reaction with building block 32 gave bisubstituted triazine 36; the remaining chlorine was then substituted using an excess of isobutylamine. Removal of the Fmoc and Boc groups and subsequent capping with  $\text{Ac}_2\text{O}$  or mesyl chloride, gave compounds 38 and 39 respectively.

The solid phase synthesis proceeded by selective removal of the trityl group, followed by a reaction with 32 which elongated the triazine-piperazine chain. The additional triazine was functionalised by a reaction with amyl amine. The synthesis was concluded by a reaction of the di-triazine with cyanuric chloride followed by ring closure and a final reaction with a third amine, after removal of the Boc group, to provide compound 44. In addition, a combinatorial library of 48 macrocyclic compounds was synthesised. Diversity was obtained by functionalisation of the triazines using various commercially available amines.

The solid phase synthesis is an efficient way to obtain a library of macrocycles that may act as receptor molecules towards a variety of complementary ligands.

### **Example 3 Preparation of macrocyclic compounds incorporating xylenediamine spacer groups**

To further exemplify the invention, other diamines such as xylenediamine were used to create novel macrocyclic structures. For the preparation of such

xylenediamine-triazine macrocycles, a new building block **46** was required and easily prepared from monoprotected xylenediamine **45** and cyanuric chloride in 85% yield, as illustrated in Scheme 7. After introduction of orthogonally protected linker **47** (96% yield), the remaining chlorine in triazine **48** was substituted using an excess of  
5 amylamine to afford triazine **49** in 98% yield. Compound **49** can be regarded as a starting point for the preparation of xylene-triazine oligomers.

Monotriazine **49** was elongated by subsequent reactions with TFA and building block **46** to give bis-triazine **50** in 83% yield. Refluxing this compound with an excess of either amyl- or iso-butylamine afforded oligomers **51a** and **51b** both in 97% yield. In  
10 order to obtain precursors to macrocycles containing three triazine moieties, the Z-group from compounds **51** was removed and the resulting free amine was subsequently used in a reaction with cyanuric chloride. The crude dichlorotriazines obtained were used directly for cyclisation to afford macrocycles **52a** and **52b**. Yields of 36% and 34%, respectively were obtained for the cyclisation affording rings **52a** and **52b**. These lower  
15 yields are probably the result of the presence of rotamers, not all of which allow for cyclisation. The obtained macrocycles were further functionalised by refluxing in the presence of either amylamine or benzylamine to afford **53a** and **53b** in 71% and 72% yield respectively. The symmetric nature of **53b** was reflected in its NMR spectrum, showing only one set of signals for the three amyl groups present. Furthermore, the  
20 macrocycles possess considerable conformational freedom. This leads to interconverting (on the NMR time scale) rotamers, as is evidenced by the broad signals for all hydrogens that sharpen upon heating from 25° to 50°C.

#### **Example 4 Binding of cyanuric acid to tris-(xylenediamine-triazine) macrocycles**

On the basis of molecular modelling studies, it was anticipated that compounds  
25 **53a** and **53b** should be able to completely encircle one molecule of cyanuric acid. To verify this expectation, compound **53b** was titrated with 0.1 to 2 equivalents of cyanuric acid. After the addition of the appropriate amount of cyanuric acid, each sample was sonicated for three hours. Compound **53b** was able to dissolve at least one equivalent  
30 of cyanuric acid in CDCl<sub>3</sub>, which was otherwise poorly soluble in this solvent. The addition of cyanuric acid to compound **53b** generates new signals in the NMR spectrum that were assigned to the newly-formed complex. The equilibrium established between the free and the bound state is slow on the NMR time scale, as both the free and bound state were visible. A binding constant of  $2.5 \times 10^4 \text{ M}^{-1}$  was calculated by non-linear



regression of binding isotherms. The curve obtained was consistent with the postulated 1:1 stoichiometry.

Remarkably, the addition of cyanuric acid to **53a** resulted in a second spot on TLC ( $R_f = 0.85$ ; 10% methanol in dichloromethane), moving faster than pure **53a** ( $R_f = 0.25$ ). The spot for uncomplexed **53a** completely disappeared after titrating the receptor with excess cyanuric acid, providing further evidence for the stability of the complex and the slow kinetics of the equilibrium. To test the selectivity of **53b** towards cyanuric acid, attempts were made to bind analogues such as thymine, uracil and 5-bromouracil. However, none of these compounds were soluble in  $\text{CDCl}_3$  in the presence of compound **53b** indicating a lack of affinity for the macrocycle.

**Example 5 Binding of octyl glycosides to tris(xylenediamine-triazine) macrocycles**

There is circular presentation of hydrogen bond donors and acceptors in the macrocycles. This was examined further, by interaction with octyl glycosides. Preliminary molecular modelling studies suggested that the size of the ring is such that it could provide for hydrogen bond donors and acceptors complementary to those of pyranosides. Both compounds **53a** and **53b** were titrated with 1-O-n-octyl- $\alpha$ -D-glucopyranoside, 1-O-n-octyl- $\beta$ -D-glucopyranoside and 1-O-n-octyl- $\beta$ -D-galactopyranoside. In these experiments, the complexation-induced shift of the anomeric proton was observed, on increasing the number of equivalents of receptor molecule from 0.1 to 8. In all cases, an upfield complexation induced shift was observed for the anomeric proton of the glycosides, and all the binding isotherms matched well with the formation of 1:1 complexes. This was confirmed for all combinations by Job plots which had their maximum at a mole fraction of 0.5 for octyl- $\beta$ -glucopyranoside and macrocycle **53b**. Assuming 1:1 stoichiometry for these complexes, binding constants of  $2.5\text{--}7.6 \times 10^3 \text{ M}^{-1}$  were calculated using non-linear regression. Macrocycles **53a** and **53b** showed similar selectivities, both favouring octyl- $\beta$ -glucopyranoside.

Specific reactions will now be described, in the following experimental section.

Reactions were carried out at ambient temperature unless stated otherwise. TLC analysis was performed on polyester pre-coated silicagel (250 mm, 5-17 mm) plates. Spots were visualised with UV light, ninhydrin (0.3 g in 100 mL HOAc/n-BuOH 3:97 v/v) or  $\text{Cl}_2$ -TDM. Column chromatography was performed on Merck kieselgel 60 (40-63  $\mu\text{m}$ ) and flash column chromatography on Merck kieselgel 60H (5-40  $\mu\text{m}$ , applying 1 bar pressure).  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded with a Jeol Lambda 400

spectrometer (399.65 MHz for  $^1\text{H}$ , chemical shift values are given in ppm relative to TMS; 100.4 MHz for  $^{13}\text{C}$ , chemical shift values are given in ppm relative to  $\text{CDCl}_3$  ( $\delta = 77.0$  ppm) or  $[\text{D}_6]\text{DMSO}$  ( $\delta = 39.5$  ppm)). The temperature was 298K ( $\pm 0.5$  K) for all experiments unless stated otherwise. Electron Spray Mass Spectrometry (ESI), Fast Atom Bombardment (FAB) and Liquid secondary Ion Mass Spectrometry (LSIMS) were carried out on a Bruker Bio-Apex II FT-ICR, Micromass Q-TOF or MSI Concept.

### Compound 2

To a solution of 3.44 g piperazine (39.9 mmol) in 100 mL DCM, a solution of 4.37 g  $\text{Boc}_2\text{O}$  (20.0 mmol) in 50 mL DCM was added over a period of three hours. The mixture was allowed to stir 22 hours before the solvent was evaporated. To the residue 100 mL water was added and the insoluble product was removed by filtration. The aqueous solution was extracted with three portions of 100 mL DCM and the combined organic layers were evaporated to afford 3.08 g Boc-piperazine (83%).

### Compound 3

A fine slurry of cyanuric chloride was prepared by adding a solution of 1.11 g cyanuric chloride (6.0 mmol) in 24 mL acetone into 36 mL of well stirred ice-water. A solution of 1.12 g Compound 2 (6.0 mmol) in 10 mL acetone and 504 mg  $\text{NaHCO}_3$  (6.00 mmol) in 10 mL water were added. After stirring the mixture for 2 hours at  $0^\circ\text{C}$  the solid was filtered off, washed with water and dried *in vacuo* over  $\text{P}_2\text{O}_5$  affording 1.93 g (96%) of product.  $R_f = 0.63$  (EtOAc/hexanes 1:1). HRMS (ESI) calcd for  $\text{C}_{12}\text{H}_{17}\text{Cl}_2\text{N}_5\text{NaO}_2$  ( $\text{M}+\text{Na}$ ) $^+$ : 356.0657, found  $m/z$ : 356.0685.

### Compound 4

To a solution of 559 mg Compound 2 (3.0 mmol) and 460 mL  $\text{Et}_3\text{N}$  (3.3 mmol) in 20 mL DCM, a solution of 433  $\mu\text{L}$  benzyl chloroformate (3.03 mmol) in 10 mL DCM was added at  $0^\circ\text{C}$ . The mixture was stirred at room temperature for 30 minutes before the solution was concentrated *in vacuo*. The residue was dissolved in EtOAc and the resulting solution was washed with 1M  $\text{KHSO}_4$  (twice), 5%  $\text{NaHCO}_3$ , brine and dried on  $\text{Na}_2\text{SO}_4$ . Evaporation of the solvent yielded 957 mg of title compound (100%).  $R_f = 0.55$  (EtOAc/hexanes 1:1).

### Compound 5

At  $0^\circ\text{C}$  10 mL of TFA was added to a solution of 4.14 g of Compound 4 (12.9 mmol) in 30 mL DCM. The mixture was stirred for 30 minutes at room temperature before it was concentrated *in vacuo*. To the residue 150 mL 1M NaOH was added and the aqueous layer was extracted with DCM (150 and 100 mL subsequently) and the

combined organic layers were dried on  $\text{MgSO}_4$  and evaporated to afford 2.85 g of 1-Z-piperazine (100%).

#### Compound 6

- 5 To a solution of 1.21 g Compound 5 (5.50 mmol) in 30 mL water, 583 mg  $\text{Na}_2\text{CO}_3$  (5.50 mmol) and a suspension of 1.67 g Compound 3 (5.00 mmol) in 10 mL acetone were added. After stirring at  $65^\circ\text{C}$  for 5 hours the white solid was filtered off and washed with water. Drying *in vacuo* over  $\text{P}_2\text{O}_5$  overnight yielded 2.51 g of product (97%).  $R_f = 0.25$  (1% MeOH in DCM). HRMS (FAB) calcd for  $\text{C}_{24}\text{H}_{33}\text{ClN}_7\text{O}_4$  ( $\text{M}+\text{H}$ ) $^+$ : 518.2283, found  $m/z$ : 518.2291.

#### Compound 7a

- A solution of 1.04 g Compound 6 (2.0 mmol) and 1159  $\mu\text{L}$  amylamine (10.0 mmol) in 20 mL THF was refluxed for 6 hours before the solvent was evaporated. The residue was dissolved in EtOAc and the resulting solution was washed with 1M  $\text{KHSO}_4$  (twice), water, 5%  $\text{NaHCO}_3$ , brine and dried on  $\text{MgSO}_4$ . Evaporation of the solvent 15 afforded 1.13 g of trisubstituted triazine (99%).  $R_f = 0.08$  (1% MeOH in DCM). HRMS (ESI) calcd for  $\text{C}_{29}\text{H}_{45}\text{N}_8\text{O}_4$  ( $\text{M}+\text{H}$ ) $^+$ : 569.35582 found  $m/z$ : 569.3562.

#### Compound 7b

- Compound 7b was prepared from Compound 6 (1.55 g, 3.0 mmol) and 2-phenylethylamine (1.88 mL, 15.0 mmol) according to the procedure described for 20 Compound 7a, except that refluxing was for 24 hours.  $R_f = 0.44$  (EtOAc/hexanes 1:1). HRMS (ESI) calcd for  $\text{C}_{32}\text{H}_{42}\text{N}_8\text{O}_4$  ( $\text{M}+\text{H}$ ) $^+$ : 603.3407 found  $m/z$ : 603.3409

#### Compound 9

- To a solution of 989 mg Compound 7a (1.74 mmol) in 6 mL DCM, 2 mL TFA was 25 added and the mixture was stirred for 30 minutes before it was evaporated. The residue was coevaporated with THF three times. The residue was redissolved in 30 mL THF and 485  $\mu\text{L}$   $\text{Et}_3\text{N}$  (3.48 mmol) and 581 mg Compound 3 (1.74 mmol) were added. The mixture was stirred for 2 hours at  $40^\circ\text{C}$  and kept basic by the addition of  $\text{Et}_3\text{N}$  before the volatiles were removed *in vacuo*. The residue was taken up in EtOAc and the resulting 30 solution was washed with 1M  $\text{KHSO}_4$  (twice), water, 5%  $\text{NaHCO}_3$ , brine and dried on  $\text{MgSO}_4$ . Evaporation of the solvent and column chromatography (eluent: EtOAc/hexanes 1:2) afforded 1.33 g of product (100%).  $R_f = 0.51$  (EtOAc/hexanes 1:1). HRMS (ESI) calcd for  $\text{C}_{36}\text{H}_{53}\text{ClN}_{13}\text{O}_4$  ( $\text{M}+\text{H}$ ) $^+$ : 766.4032 found  $m/z$ : 766.4028.

#### Compound 10

Compound 10 was prepared from Compound 9 (1.16 g, 1.51 mmol) and isobutylamine (665  $\mu$ L, 6.69 mmol) in 10 mL THF and refluxed overnight. The solvent was removed *in vacuo* and the residue was redissolved in EtOAc. The resulting solution was washed with 1M KHSO<sub>4</sub> (twice), water (twice), 5% NaHCO<sub>3</sub> and brine. Drying on  
5 MgSO<sub>4</sub> and evaporation of the solvent afforded 1.13 g of product (93%). *R<sub>f</sub>* 0.52 (EtOAc/Hexanes 1:1). HRMS (ESI) calcd for C<sub>40</sub>H<sub>63</sub>N<sub>12</sub>O<sub>4</sub> (M+H)<sup>+</sup>: 803.5151 found *m/z*: 803.5149.

#### Compound 11

To Compound 10 (1.10 g, 1.37 mmol) in 25 mL THF/EtOH (4:3), 350 mg of Pd/C  
10 (10%) was added and the resulting solution was stirred under a hydrogen atmosphere overnight. The catalyst was filtered off and the filtrate evaporated. The residue was dissolved in 3 mL acetone and the resulting solution was added to a freshly prepared suspension of 111 mg cyanuric chloride (0.60 mmol), precipitated from 5 mL acetone in 5 mL water, after which 50 mg NaHCO<sub>3</sub> (0.60 mmol) was added. After stirring for two  
15 hours at 0°C the aqueous suspension was extracted with DCM (twice) and the combined organic layers were dried on MgSO<sub>4</sub> and evaporated. Column chromatography (eluent: 2% MeOH in DCM) yielded 595 mg of product (61%). *R<sub>f</sub>* = 0.46 (EtOAc/hexanes 1:1). HRMS (ESI) calcd for C<sub>35</sub>H<sub>56</sub>Cl<sub>2</sub>N<sub>17</sub>O<sub>2</sub> (M+H)<sup>+</sup>: 816.4180 found *m/z*: 816.4184.

#### 20 Compound 12

Compound 12 was prepared from Compound 10 (0.40 g, 0.50 mmol) according to the procedure described for Compound 9. Column chromatography (eluent: EtOAc/hexanes 2:3) afforded 465 mg of product (93%). *R<sub>f</sub>* 0.45 (EtOAc/Hexanes 1:1). HRMS (ESI) calcd for C<sub>47</sub>H<sub>71</sub>ClN<sub>19</sub>O<sub>4</sub> (M+H)<sup>+</sup>: 1000.5625 found *m/z*: 1000.5649.

#### 25 Compound 13

A solution of 0.42 g Compound 12 (0.42 mmol) and 239  $\mu$ L cyclohexylamine (2.09 mmol) in 5 mL THF was refluxed for 20 hours. The solvent was removed *in vacuo* and the residue was redissolved in EtOAc. The resulting solution was washed with water (acidified to pH 2 with 1M HCl), water (twice), 5% NaHCO<sub>3</sub>, brine, dried on MgSO<sub>4</sub>  
30 and evaporated, which afforded 413 mg of product (92%). *R<sub>f</sub>* 0.40 (EtOAc/Hexanes 1:1). HRMS (ESI) calcd for C<sub>59</sub>H<sub>82</sub>N<sub>20</sub>NaO<sub>4</sub> (M+Na)<sup>+</sup>: 1085.6726, found *m/z*: 1085.6744.

#### Compound 14

To a solution of 0.35 g Compound 13 (0.33 mmol) in 20 mL THF/EtOH (1:1), 200 mg of Pd/C (10%) was added and the resulting solution was stirred under a

hydrogen atmosphere overnight. The catalyst was filtered off and the filtrate evaporated. The residue was dissolved in 2 mL acetone and the resulting solution was added to a freshly prepared suspension of 61 mg cyanuric chloride (0.33 mmol), precipitated from 4 mL acetone in 6 mL water, after which 28 mg NaHCO<sub>3</sub> (0.33 mmol) was added. After stirring for two hours at 0°C the aqueous suspension was extracted with DCM (three times) and the combined organic layers were dried on MgSO<sub>4</sub> and evaporated. Column chromatography (eluent: EtOAc/hexanes 1:2) yielded 227 mg of the title compound (68%). *R<sub>f</sub>* 0.52 (EtOAc/hexanes 1:1). HRMS (ESI) calcd for C<sub>48</sub>H<sub>76</sub>Cl<sub>2</sub>N<sub>23</sub>O<sub>2</sub> (M+H)<sup>+</sup>: 1076.5924, found *m/z*: 1076.5945.

#### 10 Compound 15

To a solution of 0.36 g of Compound 13 (0.34 mmol) in 6 mL DCM, 2 mL TFA was added and the mixture was stirred for 30 minutes before it was evaporated. The residue was coevaporated with THF three times. The residue was redissolved in 10 mL THF and 95 µL Et<sub>3</sub>N (0.68 mmol) and 0.11 g Compound 3 (0.34 mmol) were added. The mixture was stirred for 2 hours at 40°C and kept basic by the addition of Et<sub>3</sub>N before the volatiles were removed *in vacuo*. The residue was taken up in EtOAc and the resulting solution was washed with water (acidified to pH 2 with 1M HCl), water (twice), 5% NaHCO<sub>3</sub>, brine and dried on MgSO<sub>4</sub>. Evaporation of the solvent and column chromatography (eluent: EtOAc/hexanes 2:3) afforded 378 mg of product (88%). *R<sub>f</sub>* 0.45 (EtOAc/Hexanes 1:1). *R<sub>f</sub>* 0.42 (EtOAc/hexanes 1:1). HRMS (ESI) calcd for C<sub>60</sub>H<sub>90</sub>ClN<sub>25</sub>NaO<sub>4</sub> (M+Na)<sup>+</sup>: 1282.7194, found *m/z*: 1282.7246.

#### Compound 16

Compound 15 was functionalised (0.42 g, 0.33 mmol) according to the procedure described for Compound 12 except that cyclohexylamine was replaced by aniline and the reaction time was extended to 36 hours, to afford 410 mg of the title compound (93%). *R<sub>f</sub>* 0.38 (EtOAc/hexanes 1:1). HRMS (ESI) calcd for C<sub>66</sub>H<sub>97</sub>N<sub>26</sub>O<sub>4</sub> (M+H)<sup>+</sup>: 1317.8157, found *m/z*: 1317.8145.

#### Compound 17

Compound 17 was prepared from Compound 16 (0.13 g, 0.33 mmol) according to the procedure described for Compound 14, except that the reaction time was 9 hours, to yield 74 mg of compound 17 (56%). *R<sub>f</sub>* 0.47 (EtOAc/hexanes 1:1). HRMS (ESI) calcd for C<sub>61</sub>H<sub>90</sub>Cl<sub>2</sub>N<sub>29</sub>O<sub>2</sub> (M+H)<sup>+</sup>: 1330.7204, found *m/z*: 1330.7208.

#### Compound 18

Compound 18 was prepared from Compound 16 (0.11 g, 84 mmol) according

to the procedure described for Compound 15. Column chromatography (eluent: 2.5% MeOH in DCM) afforded 89 mg of the title compound (70%).  $R_f$  0.20 (2.5% MeOH in DCM).

#### Compound 19

- 5           Compound 19 was prepared from Compound 18 (0.24 g, 0.16 mmol) and 2-phenylethylamine (303  $\mu$ L, 2.42 mmol) according to the procedure described for Compound 12, except that the reaction time was 5 hours, to yield 241 mg of product (94%).  $R_f$  0.17 (3% MeOH in DCM), 0.72 (EtOAc/hexanes 2:1). HRMS (ESI) calcd for  $C_{81}H_{114}N_{32}NaO_4$  (M+Na) $^+$ : 1621.9598, found  $m/z$ : 1621.9646.

#### 10   Compound 20

- Compound 20 was prepared from Compound 19 (0.14 g, 86 mmol) according to the procedure described for Compound 11, except that the reaction time was 17 hours. Column chromatography (eluent: 2.3% MeOH in DCM) afforded 104 mg of product (79%).  $R_f$  0.33 (3% MeOH in DCM). HRMS (ESI) calcd for  $C_{76}H_{108}Cl_2N_{35}O_2$  (M+H) $^+$ : 1612.8796, found  $m/z$ : 1612.8746.

#### Compound 22

- A solution of 0.17 g Compound 11 (0.21 mmol) in 25 mL 4M HCl in dioxane was stirred for two hours. The volatiles were removed *in vacuo* and the residue was coevaporated with THF twice. The intermediate was dried *in vacuo* in a desiccator on  
20   KOH for one hour. Subsequently, the intermediate was dissolved in 75 mL DMF and to the resulting solution, a solution of 0.47 mL  $Et_3N$  (3.4 mmol) in 50 mL DMF was added dropwise at 45°C. After continuing stirring for 30 minutes at this temperature the solvent was removed *in vacuo*. The residue was dissolved in DCM and the resulting solution was washed with 1M HCl, water and dried on  $MgSO_4$ . Column chromatography  
25   (eluent: 3% MeOH in DCM) afforded 115 mg of Compound 22 (69%).  $R_f$  0.52 (3% MeOH in DCM). HRMS (ESI) calcd for  $C_{30}H_{47}ClN_{17}$  (M+H) $^+$ : 680.3883 found  $m/z$ : 680.3851.

#### Compound 23

- A solution of 0.10 g Compound 12 (94 mmol) in 10 mL 4M HCl in dioxane was  
30   stirred for four hours. The volatiles were removed *in vacuo* and the residue was coevaporated with THF twice. The intermediate was dried *in vacuo* in a desiccator on KOH overnight. Subsequently, the intermediate was dissolved in 45 mL DMF and to the resulting solution, a solution of 0.13 mL  $Et_3N$  (0.94 mmol) in 5 mL DMF was added dropwise at 45°C. After continuing stirring for 30 minutes at this temperature the solvent

was removed *in vacuo*. The residue was dissolved in DCM and the resulting solution was washed with water (twice), brine and dried on  $\text{MgSO}_4$ . Column chromatography (eluent: 3% MeOH in DCM) afforded a mixture of Compound 23 and its corresponding dimer. These were separated by gel permeation chromatography (eluent: DCM/MeOH 2:1) to afford 20 mg of Compound 23 (23%).  $R_f$  0.20 (3% MeOH in DCM). HRMS (LSIMS) calcd for  $\text{C}_{43}\text{H}_{67}\text{ClN}_{23}$  ( $\text{M}+\text{H}$ )<sup>+</sup>: 940.5633 found  $m/z$ : 940.5723.

Additionally, 27 mg dimer was isolated (31%):  $R_f$  0.17 (3% MeOH in DCM). HRMS (LSIMS) calcd for  $\text{C}_{86}\text{H}_{133}\text{Cl}_2\text{N}_{46}$  ( $\text{M}+\text{H}$ )<sup>+</sup>: 1880.1193 found  $m/z$ : 1880.1048.

#### Compound 24

A solution of 72 mg Compound 17 (54 mmol) in 6 mL TFA/DCM 1:1 was stirred for one hour. The volatiles were removed *in vacuo* and the residue was coevaporated with THF twice. The intermediate was dried *in vacuo* in a desiccator on KOH overnight. Subsequently, the intermediate was dissolved in 10 mL DMF and to the resulting solution 75  $\mu\text{L}$   $\text{Et}_3\text{N}$  (0.54 mmol) was added dropwise at 45°C. After continuing stirring for 30 minutes at this temperature the solvent was removed *in vacuo*. The residue was dissolved in DCM and the resulting solution was washed with water (twice), brine and dried on  $\text{MgSO}_4$ . Column chromatography (eluent: 2.5% MeOH in DCM) afforded 38 mg of macrocyclic Compound 24 (59%). The crude product was used without further purification to prepare Compound 28.  $R_f$  0.32 (3% MeOH in DCM). HRMS (ESI) calcd for  $\text{C}_{56}\text{H}_{81}\text{ClN}_{29}$  ( $\text{M}+\text{H}$ )<sup>+</sup>: 1194.6918, found  $m/z$ : 1194.6938.

#### Compound 25

A solution of 0.10 g Compound 20 (64 mmol) in 6 mL TFA/DCM 1:1 was stirred for three hours. The volatiles were removed *in vacuo* and the residue was coevaporated with THF three times. The intermediate was dried *in vacuo* in a desiccator on KOH overnight. Subsequently, the intermediate was dissolved in 15 mL DMF and the resulting solution was added dropwise to a solution of 89  $\mu\text{L}$   $\text{Et}_3\text{N}$  (0.64 mmol) in 15 mL DMF at 45 °C. Stirring was continued for 45 minutes at this temperature before the solvent was removed *in vacuo*. The residue was dissolved in DCM and the resulting solution was washed with water (twice) and brine. A precipitate formed in the aqueous layer, which was extracted with DCM until it disappeared. The combined organic layers were dried on  $\text{MgSO}_4$  and evaporated. Column chromatography (eluent: 3% MeOH in DCM) afforded 78 mg of macrocycle 17 (83%).  $R_f$  0.57 (4% MeOH in DCM).

#### Compound 26

A solution of 0.13 g Compound 22 (0.19 mmol) and 204 mL benzylamine (1.87

mmol) in 4 mL THF was refluxed for 36 hours. The solvent was evaporated and the residue taken up in DCM. The resulting solution was washed with 1M HCl, water (three times), 5% NaHCO<sub>3</sub>, dried on MgSO<sub>4</sub> and evaporated. Column chromatography (eluent: 4% MeOH in DCM) afforded 110 mg of Compound 22 (78%). *R<sub>f</sub>* 0.30 (4% MeOH in DCM). HRMS (ESI) calcd for C<sub>37</sub>H<sub>55</sub>N<sub>18</sub> (M+H)<sup>+</sup>: 751.4852 found *m/z*: 751.4873.

#### Compound 27

A solution of 5.0 mg Compound 23 (5.3 μmol) and 20 μL benzylamine (0.16 mmol) in 1.5 mL THF was refluxed for 24 hours. The solvent was evaporated and the residue taken up in DCM. The resulting solution was washed with 1M HCl, water (twice), 5% NaHCO<sub>3</sub>, brine, dried on MgSO<sub>4</sub> and evaporated. Column chromatography (eluent: 3% MeOH in DCM) afforded 4.0 mg of Compound 27 (74%). *R<sub>f</sub>* 0.46 (5% MeOH in DCM). HRMS (ESI) calcd for C<sub>51</sub>H<sub>76</sub>N<sub>24</sub> (M+H)<sup>+</sup>: 1025.6763 found *m/z*: 1025.6696.

#### Compound 28

A solution of 35 mg Compound 24 (29 μmol) and 55 μL benzylamine (0.44 mmol) in 2 mL THF was refluxed for 6 hours. The solvent was removed *in vacuo* and the residue was redissolved in EtOAc. The resulting solution was washed with water (acidified to pH 4 with 1M HCl), water, 5% NaHCO<sub>3</sub>, brine, dried on MgSO<sub>4</sub> and evaporated. Gel permeation chromatography (eluent: DCM/MeOH 2:1) afforded 30 mg of product (80%). *R<sub>f</sub>* 0.52 (4% MeOH in DCM). HRMS (LSIMS) calcd for C<sub>64</sub>H<sub>91</sub>N<sub>30</sub> (M+H)<sup>+</sup>: 1279.8043, found *m/z*: 1279.8091.

#### Compound 29

A solution of 74 mg Compound 25 (50 μmol) and 82 μL benzylamine (0.75 mmol) in 3 mL THF was refluxed for 36 hours and stirred at room temperature overnight. The solvent was removed *in vacuo* and the residue was redissolved in DCM. The resulting solution was washed with water (acidified with 0.6 mL 1M HCl), water (three times), dried on MgSO<sub>4</sub> and evaporated. Column chromatography (eluent: 3.5 % MeOH in DCM) followed by gel permeation chromatography (eluent: DCM/MeOH 2:1) afforded 57 mg of Compound 29 (74%). *R<sub>f</sub>* 0.37 (4% MeOH in DCM). HRMS (ESI) calcd for C<sub>78</sub>H<sub>107</sub>N<sub>36</sub> (M+H)<sup>+</sup>: 1547.9474, found *m/z*: 1547.9496.

#### Compound 45

Compound 45 was synthesised according to published methods. The product was purified further by column chromatography (eluent: 30% MeOH in DCM). 11 mL xylenediamine and 2.18 g Boc<sub>2</sub>O (10.0 mmol) afforded 1.69 g (72%) of title compound.



**Compound 46**

Compound 46 was prepared from Compound 45 (1.59 g, 6.73 mmol) according to the procedure described for Compound 3, except that the reaction time was 3 hours, to afford 2.20 g of product (85%).  $R_f$  0.57 (EtOAc/hexanes 1:1).

**5 Compound 47**

Compound 47 was prepared from Compound 45 (1.57 g, 6.64 mmol) according to the procedures described for Compound 4 and Compound 5, to give 1.36 g product (94%).  $R_f$  0.18 (10% MeOH and 1% Et<sub>3</sub>N in DCM).

**Compound 48**

10 To a solution of 807 mg Compound 47 (2.99 mmol) in 20 mL water/acetone 1:1, 316 mg Na<sub>2</sub>CO<sub>3</sub> (2.99 mmol) and a solution of 1.04 g monosubstituted triazine 20 (2.71 mmol) in 7 mL acetone were added. After stirring at 65°C for 5 hours the suspension was cooled to room temperature and the white solid filtered off and washed with water. Drying *in vacuo* over P<sub>2</sub>O<sub>5</sub> overnight yielded 1.60 g of product (96%).  $R_f$  0.64 (10% MeOH in DCM). HRMS (ESI) calcd for C<sub>32</sub>H<sub>36</sub>ClN<sub>7</sub>NaO<sub>4</sub> (M+Na)<sup>+</sup>: 640.2415, found  $m/z$ : 640.2446.

**Compound 49**

Compound 49 was prepared from Compound 48 (1.00 g, 1.62 mmol) according to the procedure described for Compound 7a, to afford 1.06 g of the title compound 20 (98%).  $R_f$  0.66 (10% MeOH in DCM). HRMS (ESI) calcd for C<sub>37</sub>H<sub>49</sub>N<sub>8</sub>O<sub>4</sub> (M+H)<sup>+</sup>: 669.3877, found  $m/z$ : 669.3855.

**Compound 50**

Compound 50 was prepared from Compound 49 (1.03 g, 1.54 mmol) and Compound 26 (563 mg, 1.54 mmol) according to the procedure described for 25 Compound 9, except that the reaction time was extended to 4 hours. Column chromatography (eluent: 4% MeOH in DCM) afforded 1.17 g of product (83%).  $R_f$  0.50 (10% MeOH in DCM). HRMS (ESI) calcd for C<sub>48</sub>H<sub>59</sub>ClN<sub>13</sub>O<sub>4</sub> (M+H)<sup>+</sup>: 916.4501, found  $m/z$ : 916.4511.

**Compound 51a**

30 Compound 51a was prepared from Compound 50 (0.50 g, 0.52 mmol) and isobutylamine (414 mL, 4.17 mmol) according to the procedure described for Compound 10, to afford 500 mg of the title compound (97%).  $R_f$  0.47 (10% MeOH in DCM). HRMS (LSIMS) calcd for C<sub>52</sub>H<sub>69</sub>N<sub>14</sub>O<sub>4</sub> (M+H)<sup>+</sup>: 953.5626, found  $m/z$ : 953.5637.

**Compound 51b**

Compound 51b was prepared from Compound 50 (0.54 g, 0.59 mmol) and amylamine (543  $\mu$ L, 4.69 mmol) according to the procedure described for Compound 10, to afford 550 mg of the title compound (97%).  $R_f$  0.53 (10% MeOH in DCM). HRMS (ESI) calcd for  $C_{53}H_{71}N_{14}O_4$  (M+H)<sup>+</sup>: 967.5783, found  $m/z$ : 967.5748.

**Compound 52a**

To a solution of 0.24 g Compound 51a (0.25 mmol) in 14 mL THF/EtOH (1:1), 250 mg of Pd/C (10%) was added and the resulting solution was stirred under a hydrogen atmosphere overnight. After the addition of another 250 mg of Pd/C and stirring for 8 hours, the catalyst was filtered off and the filtrate evaporated. The residue was dissolved in 2 mL acetone and the resulting solution was added to a freshly prepared suspension of 44 mg cyanuric chloride (0.24 mmol), precipitated from 2 mL acetone in 4 mL water, after which 21 mg  $NaHCO_3$  (0.25 mmol) was added. After stirring for 30 minutes at 0 °C the aqueous suspension was extracted with DCM and the organic layer was dried on  $MgSO_4$  and evaporated. Flash column chromatography (eluent: 5% MeOH in DCM) yielded 106 mg of product (44%). The crude product was used directly to prepare macrocyclic Compound 52a. A solution of 55 mg precursor (57  $\mu$ mol) in 5 mL 4M HCl in dioxane was stirred for six hours. The volatiles were removed *in vacuo* and the residue was coevaporated with THF twice. The intermediate was dried *in vacuo* in a desiccator on KOH overnight. Subsequently, the intermediate was dissolved in 25 mL DMF and, to the resulting solution, a solution of 79  $\mu$ L DIPEA (0.57 mmol) in 5 mL DMF was added dropwise at 45°C. Another aliquot of DIPEA (79  $\mu$ L) was added and stirring was continued for 45 minutes at 45°C before the solvent was removed *in vacuo*. Flash column chromatography (eluent: 6% MeOH in DCM) afforded 17 mg of macrocycle Compound 52a (36%). The product was used without further purification to prepare macrocycle 53a.  $R_f$  0.25 (6% MeOH in DCM). HRMS (ESI) calcd for  $C_{42}H_{52}ClN_{17}$  (M+H)<sup>+</sup>: 830.4359, found: 830.4302.

**Compound 52b**

To a solution of 0.26 g Z-compound (0.27 mmol) in 14 mL THF/EtOH (1:1) 350 mg of Pd/C (10%) was added. After stirring the solution under a hydrogen atmosphere overnight, the catalyst was filtered off and the filtrate evaporated. The residue was dissolved in 2 mL acetone/THF (1:1) and the resulting solution was added to a freshly prepared suspension of 47 mg cyanuric chloride (0.26 mmol), precipitated from 2 mL acetone in 4 mL water, after which 23 mg  $NaHCO_3$  (0.27 mmol) was added. After

stirring for 30 minutes at 0 °C, water was added, the aqueous suspension extracted with DCM and the combined organic layers were dried on MgSO<sub>4</sub> and evaporated. Flash column chromatography (eluent: 5% MeOH in DCM) yielded 127 mg of intermediate product (48%). The crude product was used directly to prepare macrocyclic Compound 52b according to the procedure described for Compound 52a. Flash column chromatography (eluent: 4.5% MeOH in DCM) afforded 37 mg of Compound 52b (34%). The product was used without further purification to prepare Compound 53b. *R*<sub>f</sub> 0.11 (5% MeOH in DCM). HRMS (ESI) calcd for C<sub>43</sub>H<sub>54</sub>ClN<sub>17</sub> (M+H)<sup>+</sup>: 844.4509; found *m/z*: 844.4542.

#### 10 Compound 53a

A solution of 17 mg Compound 52a (20 μmol) and 22 μL benzylamine (0.20 mmol) in 3 mL THF was refluxed overnight. The solvent was removed *in vacuo* and the residue was purified by column chromatography (eluent: 10 % MeOH in DCM) followed by gel permeation chromatography (eluent: DCM/MeOH 2:1) to yield 13 mg of product (71%). *R*<sub>f</sub> 0.26 (10% MeOH in DCM). HRMS (ESI) calcd for C<sub>49</sub>H<sub>61</sub>N<sub>18</sub> (M+H)<sup>+</sup>: 901.5327, found: 901.5374.

#### Compound 53b

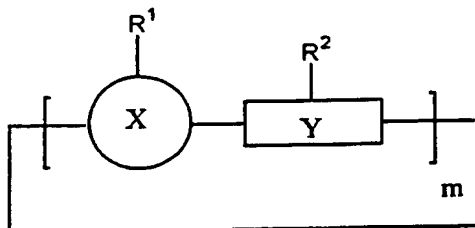
A solution of 33 mg Compound 52b (39 μmol) and 43 μL amylamine (0.39 mmol) in 2 mL THF was refluxed for 20 hours. The solvent was removed *in vacuo* and the residue was purified by flash column chromatography (eluent: 7 % MeOH in DCM) to afford 25 mg of product (72%). *R*<sub>f</sub> 0.22 (7% MeOH in DCM). HRMS (ESI) calcd for C<sub>48</sub>H<sub>67</sub>N<sub>18</sub> (M+H)<sup>+</sup>: 895.5791; found *m/z*: 895.5864.

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CLAIMS

1. A macrocyclic compound of the formula

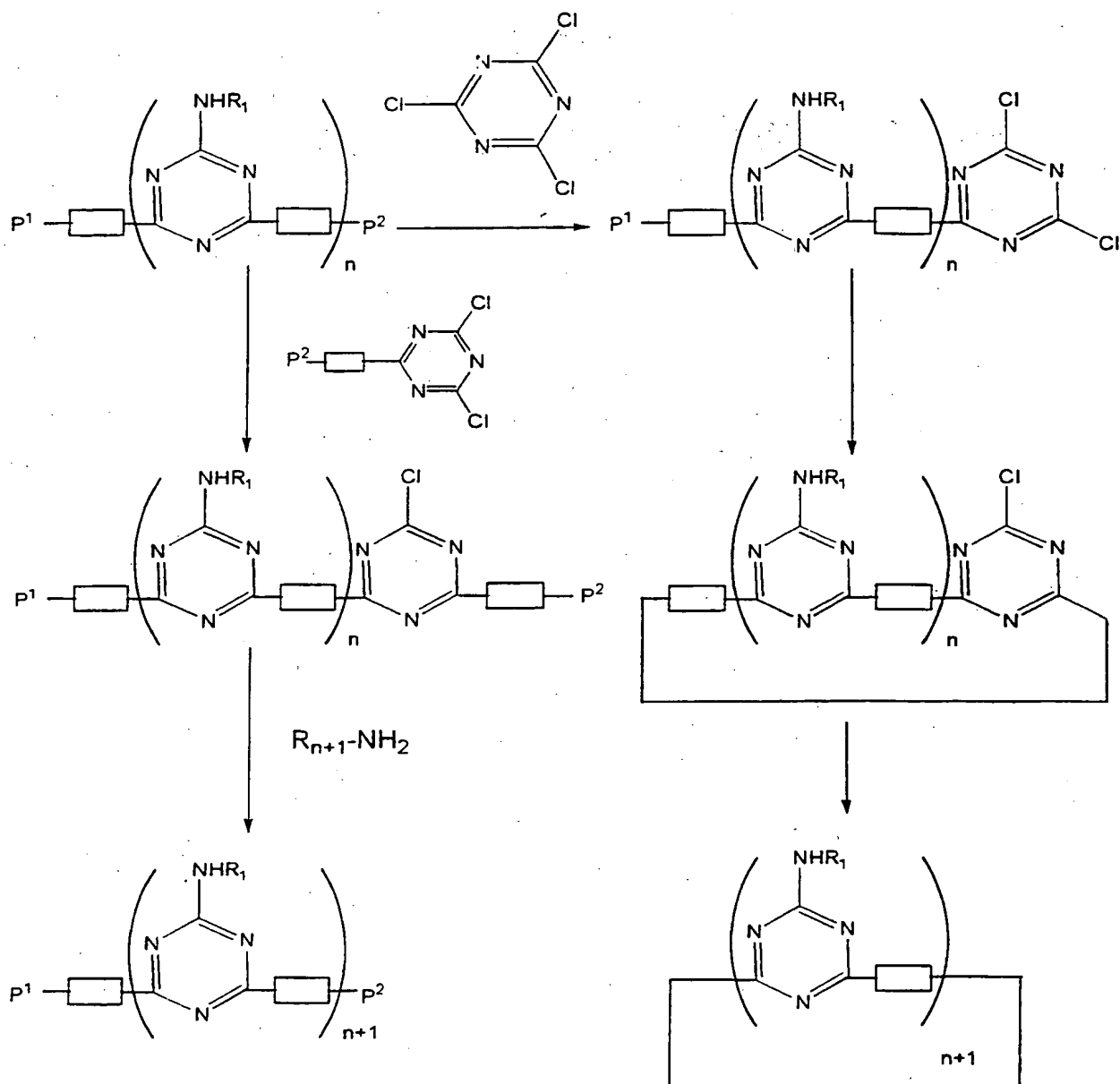
5



10 wherein X is a ring bearing an optionally-protected functional group or solid phase group R<sup>1</sup>, Y is a linker optionally bearing an optionally-protected functional group or solid phase group R<sup>2</sup>, m is at least 3, and each instance of X, Y, R<sup>1</sup> and R<sup>2</sup> may be the same or different.

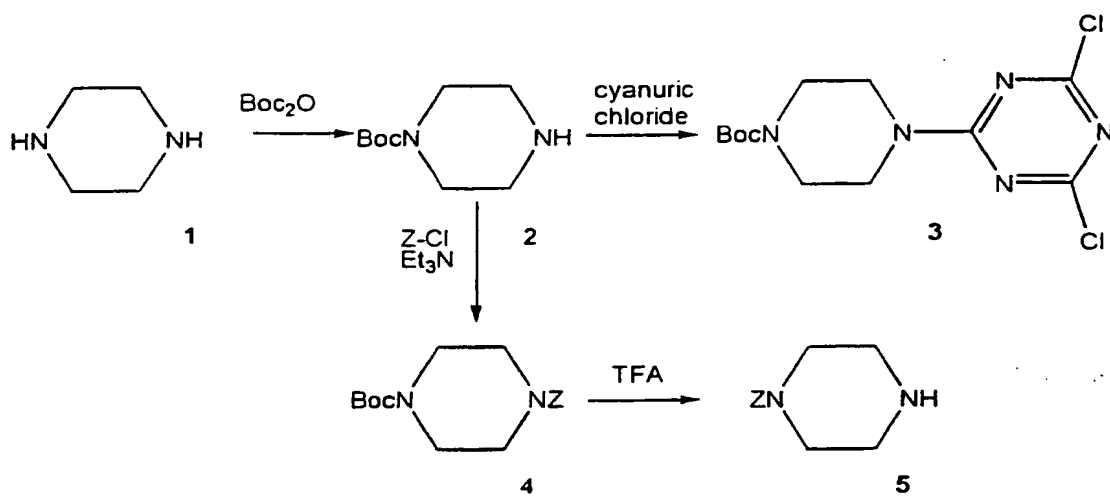
2. A compound according to claim 1, wherein each X is the same.
3. A compound according to claim 1 or 2, wherein X is a triazine ring.
- 15 4. A compound according to any preceding claim, wherein m is 3.
5. A compound according to any preceding claim, wherein Y includes a piperazine or xylene ring.
6. A compound according to any preceding claim, wherein R<sup>1</sup> or R<sup>2</sup> is a solid phase.
- 20 7. A compound according to any preceding claim, wherein R<sup>1</sup> is an optionally protected functional group.
8. Use of a compound according to any preceding claim, for the capture of a solute in a solution.
9. Use according to claim 8, for the analysis, separation, isolation, purification,
- 25 quantification, characterisation or identification of a chemical, biological or therapeutic compound.
10. A compound according to any of claims 1 to 7, for use in diagnosis or therapy.

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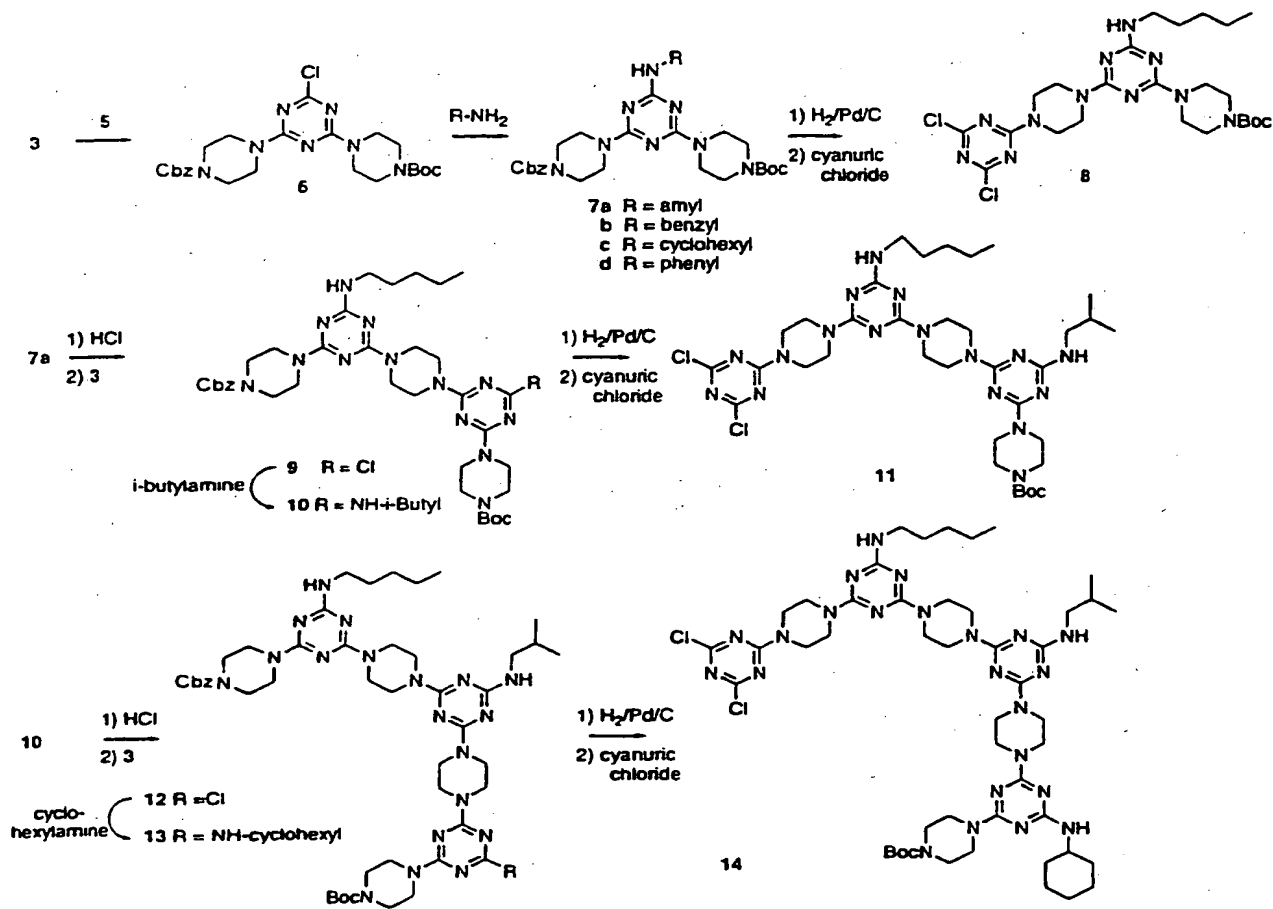
Scheme 1

2/8

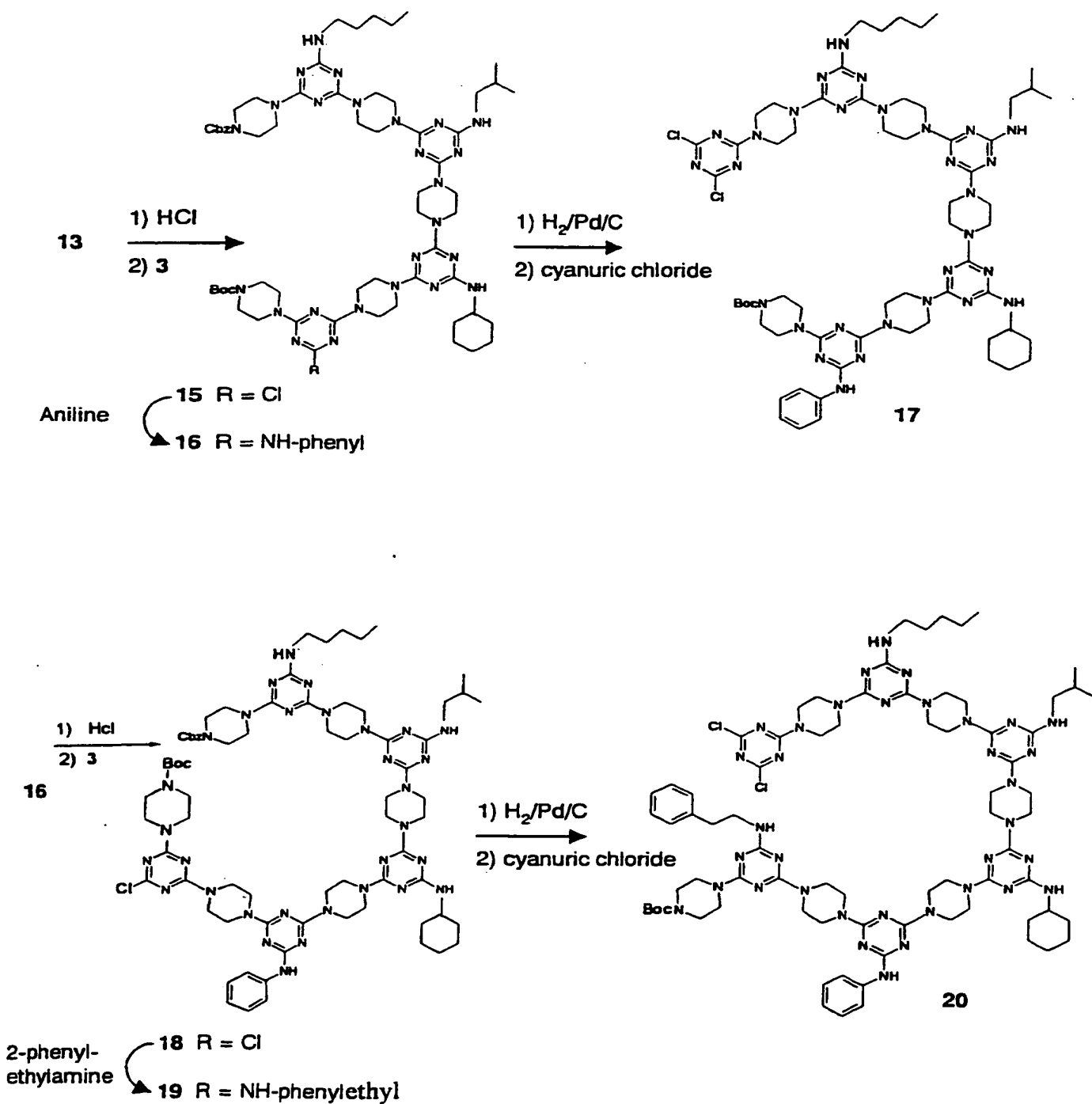


Scheme 2

3/8



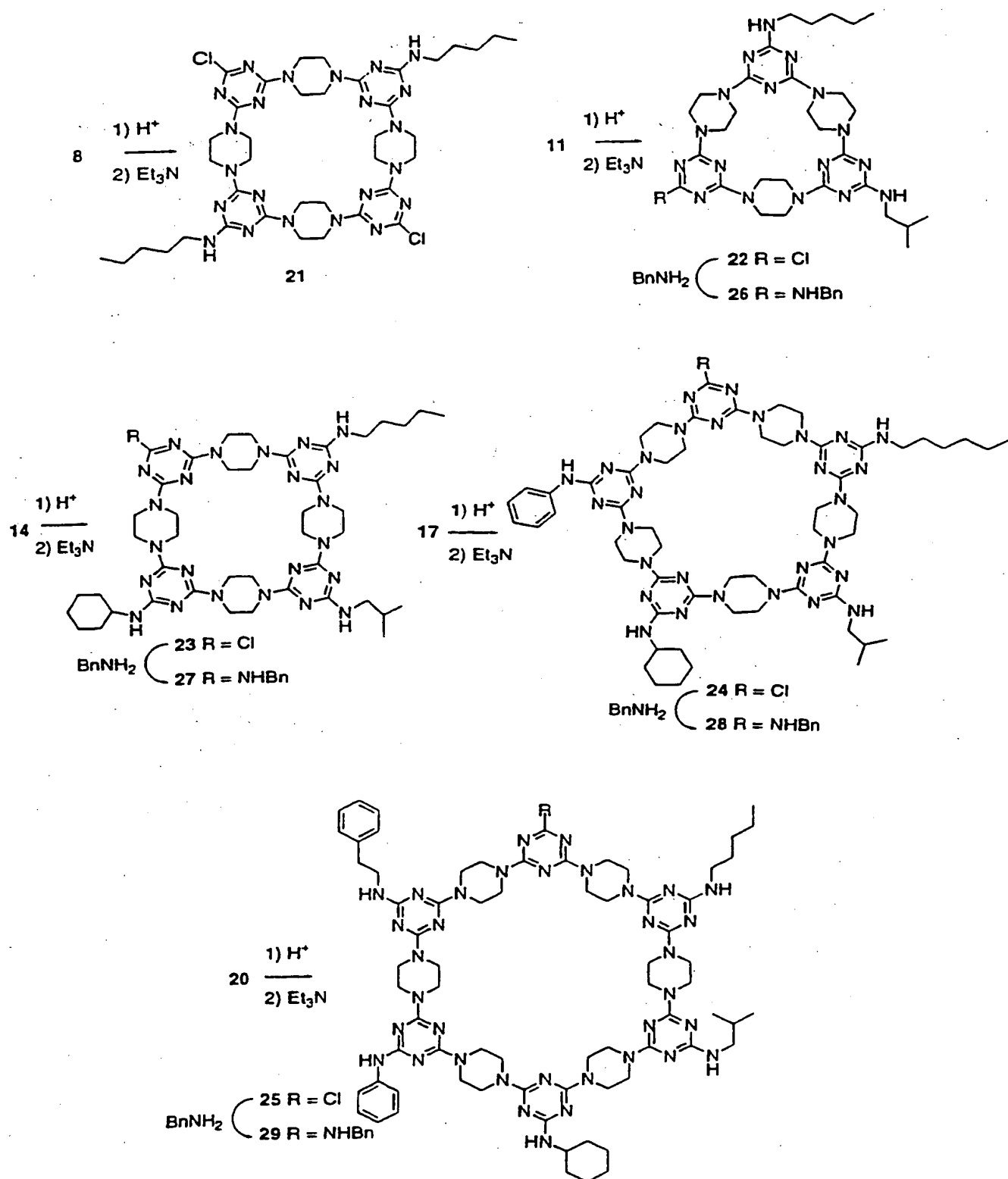
Scheme 3A



Scheme 3B: Synthesis of triazine-piperazine oligomers

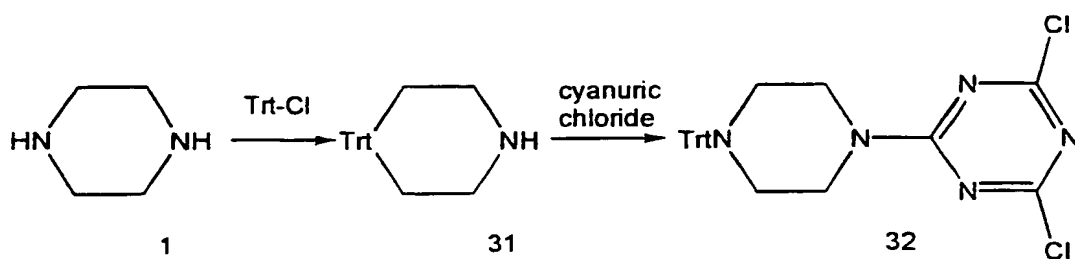


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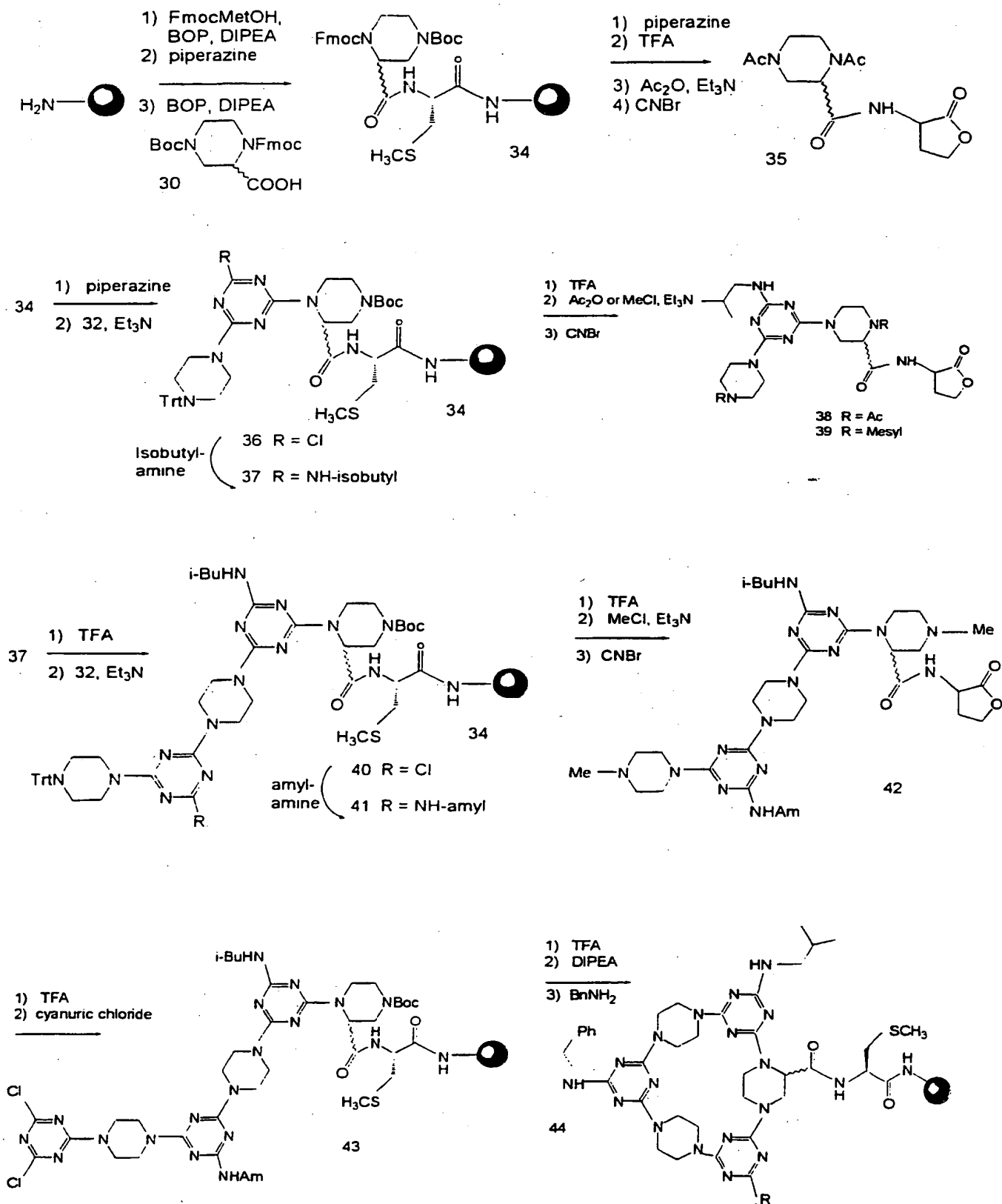
Scheme 4

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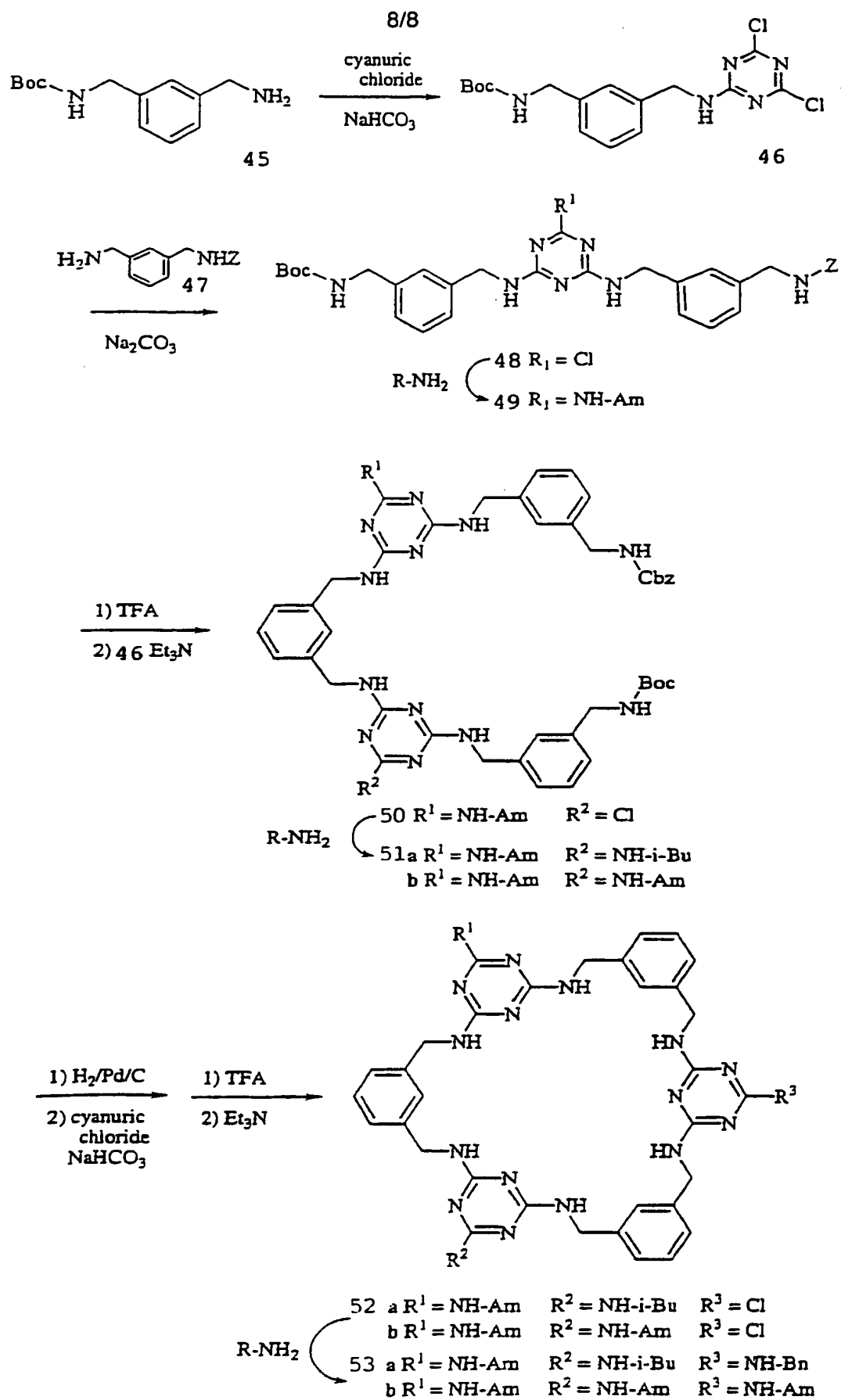


Scheme 5

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Scheme 6



Scheme 7

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/04725

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07D251/50 C07D251/54 C07D251/70 A61K31/53

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	H. GRAUBAUM ET AL.: "Synthese und Transportuntersuchungen von Dioxapolyazacalix'4!arenen und Triazino-Kronenethern" J. PRAKT. CHEM./CHEM.-ZTG., vol. 340, no. 2, 1998, pages 165-170, XP000995728 * Scheme 1 *	1-5
X	M. MASCAL ET AL.: "Synthesis and Structural Characterization of an S-Linked Calix'3!azine" TETRAHEDRON LETTERS, vol. 38, no. 43, 1997, pages 7639-7640, XP004189864 * Compound of formula 2 *	1-5



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

### \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*G\* document member of the same patent family

Date of the actual completion of the international search

20 April 2001

Date of mailing of the international search report

18. 05. 01

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Authorized officer

Herz, C

# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 00/04725

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	C.-M. SHU ET AL.: "Synthesis of Calix[4]arenes with Four Different "Lower Rim" Substituents" J. ORG. CHEM., vol. 64, no. 8, 1999, pages 2673-2679, XP002165630 cited in the application figures 1,2 ---	1-5
X	EP 0 560 567 A (TEXACO DEVELOPMENT CORP.) 15 September 1993 (1993-09-15) claims 1-7 ---	1-5
Y	P. L. ANELLI ET AL.: "Doubly and Triply Bridged Polyoxapolyazaheterophanes Derived from 2,4,6-Trichloro-s-triazine" J. ORG. CHEM., vol. 49, no. 22, 1984, pages 4197-4203, XP000615835 cited in the application tables IV,V ---	1-10
Y	P. H. RASMUSSEN, J. REBEK, JR.: "A Pseudopeptide Platform with Side Chains Addressable for Combinatorial Applications" TETRAHEDRON LETT., vol. 40, 1999, pages 3511-3514, XP002165631 cited in the application figure 1 ---	1-10
A	P. LIPKOWSKI ET AL.: "Self-assembly of a 2 x 2 hydrogen bonded grid" CHEM. COMMUN., 1999, pages 1311-1312, XP002165632 cited in the application * Scheme 1 *	1-10
A	J. P. MATHIAS ET AL.: "Self-Assembly through Hydrogen Bonding: Peripheral Crowding - A New Strategy for the Preparation of Stable Supramolecular Aggregates Based on Parallel, Connected Ca(3).M(3) Rosettes" J. AM. CHEM. SOC., vol. 116, no. 10, 1994, pages 4326-4340, XP000942081 cited in the application figure 1 ---	1-10
	-/--	

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/04725

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	H. ISHIDA ET AL.: "Serine Proteinases: Hydrolytic Activity of Cyclic Peptides Which Include a Non-natural Amino Acid" TETRAHEDRON LETT., vol. 36, no. 49, 1995, pages 8987-8990, XP002165633 * Scheme 1 *	1-10
A	DE 39 32 914 A (SANDOZ-PATENT-GMBH) 11 April 1991 (1991-04-11) page 3 -page 5	1-10

# INTERNATIONAL SEARCH REPORT

international application No.  
PCT/GB 00/04725

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.



## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

## Continuation of Box I.2

Present claims 1 to 10 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds claimed in claims 3 and 5.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/04725

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 560567 A	15-09-1993	US 5219955 A	15-06-1993
		US 5199959 A	06-04-1993
		CA 2090950 A	10-09-1993
		JP 6016649 A	25-01-1994
		CA 2090944 A	10-09-1993
DE 3932914 A	11-04-1991	WO 9104987 A	18-04-1991
		DE 59008147 D	09-02-1995
		EP 0451228 A	16-10-1991
		JP 4502034 T	09-04-1992
		US 5493022 A	20-02-1996